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IgM, Fcμ-receptors and malarial immune evasion

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Abstract

Immunoglobulin M (IgM) is an ancestral antibody class found in all jawed vertebrates from sharks to mammals. This ancient ancestry is shared by malaria parasites (genus *Plasmodium*) that infect all classes of terrestrial vertebrates with whom they coevolved. IgM, the least studied, and most enigmatic of the vertebrate immunoglobulins has recently been shown to form an intimate relationship with the malaria parasite *Plasmodium falciparum*. Here we discuss how this association might come about, building on the recently determined structure of the human IgM pentamer, and how this interaction could affect parasite survival, particularly in light of the just discovered Fcμ-receptor (FcμR) localized to B and T cell surfaces. As this parasite may exploit an interaction with IgM to not only limit immune detection but also manipulate the immune response when detected, a better understanding of this association may prove critical for the development of improved vaccines or vaccination strategies.

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Ancient ancestry and crucial functions of IgM

IgM is the oldest and only isotype of antibody (Ab) that is expressed by all species of vertebrates. Unlike most other isotypes, there are two classes of IgM: an antigen-specific (or immune) IgM class that is produced in response to exposure to specific pathogens and a low affinity, more broadly reactive (so called “natural” or “non-immune”) IgM class that is synthesized without prior exposure to any particular antigen. This latter form, apparently designed to recognize common motifs of widespread foreign invaders as part of the first line of host defense, is likely responsible for the conservation of this isotype among distantly related vertebrates (1). Moreover, probably befitting their common ancestry, IgM molecules from all species studied to date are higher-order multimeric complexes (predominantly pentamers in human plasma), a physical property that endows the molecule with extraordinary functions, including multivalent binding to both antigens and receptors (2–4).

Natural IgM produced by peritoneal B-1 cells, is an important mediator of innate immunity controlling the dissemination of viruses and bacteria (3,4). A subset of these B-1 cells can also contribute to the production of immune IgM and provide long-term T cell-independent immunity against re-infection (5), although immune IgM can also be produced in a T cell-dependent manner by conventional B-2 cells in the spleen and lymph nodes (6). IgM constitutes about 10% of human plasma immunoglobulin and levels of IgM are approximately 25% higher in females than males (7,8), a finding that has led to the notion that males are significantly more susceptible to infections than females (9,10). Because selective IgM deficiency is rare in humans, an understanding of the importance for IgM in immunity has come from studies using secretory IgM ($IgM^{-/-}$) deficient mice (3,4,11). These studies clearly show that serum IgM primes the ensuing IgG response and that IgM is particularly effective at neutralizing and agglutinating blood pathogens, particularly those that reside within cells, by inhibiting pathogen transfer from cell to cell (11). Such findings show that IgM can have a profound impact on protective immunity and, in terms of possible mechanisms, point to the involvement of interactions between the Fc domain in IgM and effector molecules, such as complement and Fc-receptors (FcRs), in mediating at least some of this immunity.

A role of IgM in human malaria

As with IgM, malarial parasites of the genus *Plasmodium* have been found in all classes of terrestrial vertebrates, including reptiles and birds. They are thus likely a long standing vertebrate pathogen against which a vigorous immune response involving IgM would be commonly expected. Indeed, parasite-specific IgM has been shown to limit parasite replication, prime memory cell generation, and is a more potent adjuvant than *Bordetella pertussis* in experiments with murine malarias (12,13). Natural IgM is also an endogenous adjuvant for vaccine-induced protective $CD8^+$ T cell responses against intracellular parasites (14). Given these considerations it is somewhat surprising that so little work has been published looking at the role of IgM in human malaria. Natural IgM is known to bind to the surface of *Plasmodium falciparum*-infected erythrocytes via the Fc, and this has been shown to correlate with severe malaria in both laboratory strains and field isolates (15,16). Only some *P. falciparum* strains bind natural IgM, and this property is seen in parasites with specific virulence-associated adhesion phenotypes including rosetting (15), and chondroitin sulfate A (CSA)-binding linked to placental malaria infection (17,18). *P. falciparum* strains showing other common adhesion phenotypes, including CD36 and ICAM-1 binding, do not appear to bind natural IgM (15). Therefore, although non-immune IgM binding is only shown by a subset of *P. falciparum* isolates, it is linked to the most serious clinical effects of malaria. A greater understanding of the role of non-immune IgM in these host-parasite

interactions has the potential to contribute new insights and interventions against life-threatening disease.

Importance of pathogen Fc binding proteins

In order to evade Fc-mediated destruction, pathogens have evolved to produce Fc-binding proteins, and those expressed by bacteria and viruses for IgG or IgA have been intensively studied (19–21). These proteins help pathogens avoid host immune responses by preventing pathogen-specific Abs from interacting with host Fc-receptors, and therefore interfere with effector functions of Ab, such as phagocytosis and complement activation (19–21). The existence of IgM Fcμ binding proteins from pathogens is less well documented than for IgG and IgA. This might be because of difficulties in differentiating low affinity Fab'_2 mediated pathogen binding by natural IgM antibodies from *bona fide* Fc-receptor interactions. Nonetheless, IgM binding proteins have been described for several protozoa including *Toxoplasma gondii* (22) and pathogenic species of *Trypanosomatidae* (23). Recently, we provided the first detailed molecular characterization of an IgM Fc-binding protein from the malaria parasite *P. falciparum* (16).

An Fcμ-binding protein expressed by Plasmodium falciparum: PfEMP1

IgM binding by *P. falciparum* infected erythrocytes occurs via the parasite variant antigen, *P. falciparum* erythrocyte membrane protein one (PfEMP1), found on the surface of infected erythrocytes (15,16). PfEMP1 variants are encoded by *var* genes and each parasite contains 50–60 *var* genes in its genome (24,25), with only one variant being expressed on the infected erythrocyte surface at a time (26). The var gene repertoires of different *P. falciparum* isolates have very little overlap, resulting in extensive diversity among different parasite isolates (27). PfEMP1 molecules are composed of Duffy binding-like (DBL) domains classified into six types (α, β, γ, δ, ε, and X), and cysteine-rich interdomain region domains (CIDR) classified into three types (α, β, and γ)(28). Individual *var* genes differ from each other by the number and type of these domains. A number of different domains from specific PfEMP1 variants expressed by IgM-binding infected erythrocytes of different parasite strains have been shown to bind nonimmune IgM (Table 1), including our identification of IgM binding by DBL4β from the PfEMP1 variant *var1* in the TM284 isolate (16). So far it has not been possible to define a specific sequence motif within these various domains that is responsible for the ability to bind non-immune IgM.

Identifying the binding site for PfEMP1 on the IgM molecule

Using recombinant domain-swapped Abs, IgM mutants, and blocking mAbs we defined the region of the IgM molecule bound by PfEMPI (16). In particular, these experiments showed that PfEMP1 binds to the Cμ4 domain of polymeric IgM (16), at a region conserved with those on IgG and IgA recognized by Fc-binding proteins from bacteria and viruses (Fig. 1) (19–21). The binding site on IgM was also shared by five distinct parasite strains (two rosetting and three CSA binding) suggesting that diverse parasite isolates expressing distinct PfEMPI variants all bind to the same (or similar) site on the human IgM molecule. Recent work from our laboratories has shown that the laboratory isolate 3D7, which was panned on CSA to express the PfEMP1 variant *var2csa* (29), also interacts with IgM via the Cμ4 domain. In this case it is the DBL5ε domain in *var2csa* that binds IgM (Salanti *et al*, unpublished data). The binding of IgM to recombinant 3D7 DBL5ε was not inhibited by soluble CSA implying that the CSA-binding sites on the DBL domains do not overlap with those used by IgM. It should be noted that recombinant *var2csa* single domains have been shown to bind promiscuously to a number of glycosylated receptors, which the native *var2csa* does not bind (29). However, using full-length recombinant FCR3 *var2csa* we have

verified that IgM does indeed interact with the complete protein, proving validation for the results with the individual domains (Salanti *et al*, unpublished data).

Structural insights into PfEMP1-IgM interactions

The use of a similar IgM binding site by distinct PfEMP1 variants suggests the possibility of a similar structural mechanism of binding to IgM. To determine if there is indeed a common IgM interaction site on these diverse *P. falciparum* DBL domains we modeled the previously described IgM binding DBL domains (Table 1) onto known DBL structures and tried to dock these molecules into a recent model of human IgM (30). This model of IgM, based on the known bent structure of IgE and supported by direct cryo-atomic force microscopy (cryo-AFM) images, shows IgM to be a mushroom-shaped molecule, with a central region formed by the Cμ3/Cμ4 domains protruding out of the plane formed by the Cμ2/Fab domains. The residues Pro^{394}-Pro^{397} and Pro^{444}-Val^{447} in the Cμ4 domain previously implicated in PfEMPI binding are localized near the junction between two monomers (16), and can therefore form the binding pocket for PfEMPI, whilst explaining the requirement for a polymeric structure.

Although it might be expected that there are many ways by which such large proteins could possibly interact, there are in fact a number of observations that place strict constraints on potentially viable models, including the narrowness of the binding pocket (with a width of a little more than only two helices), the central disposition of the IgM-binding domains within the full PfEMPI sequence (which constrains the possible locations of the termini of the IgM-binding domain), the markedly limited solvent accessibility of these domains within the native PfEMPI (29,31,32), and the localization of ligand-binding residues in other DBL domains to the loops in sub-domain 2 (31,32).

Under these strict constraints, we were able to identify only a single plausible DBL-IgM model (Fig. 2A). In this structure, the DBL domain, orientated roughly perpendicular to the Cμ2/Fab plane, binds to the side of the central IgM stem, in between two IgM monomers that project radially (Fig. 2A and Supplementary Movie 1). The predominant contacts to the IgM molecule are mediated by loops within sub-domain 2, which directly contact the opposing residues of neighboring IgM monomers (Table 2), including those between the Pro^{444}-Val^{447} loop in IgM (16). Additional contacts away from this region involve a small helix near the DBL C-terminus (residues Gln^{429}-Glu^{437}) and the other Pro^{394}-Pro^{397} loop of IgM (16). While this model satisfies all of the aforementioned constraints, what was particularly striking about this disposition of the DBL domain was the presence and number of its charged residues that are immediately adjacent to oppositely charged residues on the IgM surface (Fig. 2B). Although these residues are not strictly conserved between different IgM-binding domains, a similar set of charged residues is found in all the characterized IgM-binding DBL domains (Table 2). Moreover, inspection of these regions in the known structures of members of the DBL family, which are either from proteins that do not bind IgM (e.g. EBA-175, and PKα-DBL) or have been shown not to bind IgM (e.g. DBL3X), reveals an absence of charged residues similar to this set (D.M. Czajkowsky and R.J. Pleass, data not shown).

Such a binding mechanism involving similarly charged but not strictly conserved residues from DBL domains of different sub-classes, which moreover are not shared by all members of any single sub-class, suggests that there was not an ancestral IgM-binding DBL domain from which these domains are derived. Instead, IgM-binding may be an emergent property of certain DBL domains that resulted from some other common trait of these domains. Interestingly the most characterized IgM-binding domains (Table 2) are located proximal to the erythrocyte plasma membrane. As a result, the possible orientations of the IgM molecule

when bound to PfEMP1 would presumably be severely limited owing to the presence of the nearby erythrocyte membrane (compared with a more membrane-distal binding). Hence, it may be important for the function of these IgM Fc-binding proteins to not just interact with IgM, but also to have it, in essence, distinctly orientated with respect to the red cell membrane. This may be necessary to either prevent or perturb the access of IgM to host effector proteins like complement C1q, or perhaps more intriguingly, to actually promote the binding of IgM to host receptors (Fig. 3).

Why do pathogens bind the Fc of IgM

Exactly why *P. falciparum* has evolved an IgM Fcμ binding protein is unknown. In fact, this interaction may be part of a more general set of interactions that have evolved between *P. falciparum* and IgM, including a recently suggested inhibitory role of IgM as a novel component of the tetraspanin network on B cells (33), and in hepatocyte invasion by sporozoites (34). However, in this brief review, we will focus on the possible functional consequences of the PfEMP1-IgM interaction. As summarized in Fig. 3, these include interference with immunological signaling and clearance mechanisms, blocking the binding of specific Abs, or as a way of enhancing infected erythrocyte sequestration in the microvasculature or placenta.

Interference with Fcμ-receptors (FcμR)

The recent identification of a *bona fide* high affinity FcμR for IgM expressed by $CD19^+$ B cells (including memory B cells), $CD4^+/CD8^+$ T cells and $CD56^+/CD3^-$ NK cells may provide the target for interference by malaria parasite DBL domains (35). Although the function of FcμR in these cells is not presently understood, ectopic expression of this receptor was found to affect cell survival and proliferation, and a role in B cell activation has been suggested (35). Infection with *P. falciparum* malaria is known to induce polyclonal B cell activation leading to a marked hypergammaglobulinemia and elevated titers of autoantibodies (36). Binding by parasite PfEMP1 proteins to IgM may therefore interfere with the interaction of IgM with the FcμR on B cells in a way that favors the induction of proliferative responses by the parasite. The benefit for the parasite of such a rampant B cell activation is not presently understood.

Intriguingly, increased antigen retention by marginal zone B cells and germinal centre formation also depend on CR1 (CD35) and CR2 (CD21), the latter being responsible for Epstein-Barr virus (EBV) entry into B cells (37). EBV infected B cells occasionally undergo malignant transformation *in vivo*, giving rise to Burkitt's lymphoma, a tumor that commonly occurs in areas of Africa hyper endemic for malaria (38,39). It may therefore be that the ability of PfEMP1 to interfere with signals induced by the FcμR could not only promote cellular proliferation, but also predispose the B cell to become malignant on infection by EBV. Since children affected by Burkitt's lymphoma often present with chronic malaria it would be interesting to determine if parasite isolates derived from these children also bind IgM.

As noted above, the FcμR is also constitutively expressed on $CD4^+$ and $CD8^+$ T cells, although its function on these cells awaits elucidation (35). The receptor may allow the T cell to interact optimally with the IgM B cell receptor (BCR) or IgM immune-complexes on B cells facilitating immunological synapse formation between the two cell types, thereby enhancing B cell activation and memory induction. It may also trigger IgM-dependent cell-mediated cytotoxicity by $CD8^+$ T cells, already known to play an important role in immunity against liver stage parasites (40). The ability of PfEMP1 to bind IgM could significantly interfere with these FcμR functions and provide a significant advantage to a parasite under

attack from parasite-specific or natural IgM. These possibilities now need to be investigated for IgM and malaria.

Interference with Fcα/μ-receptors

A human receptor for IgM (and IgA), the Fcα/μR, closely related to the polymeric Ig receptor (plgR) in its ligand-binding domain, has also been described (41–43). Expressed predominantly on follicular dendritic cells (FDCs), the Fcα/μR is believed to function in antigen presentation and B-cell selection in the germinal centre response (35,44). Recent work in Fcα/μR knockout mice suggest that the receptor negatively regulates T-independent antigen (common in malaria) retention by FDCs, culminating in suppression of humoral immune responses against T-independent antigens (45). The receptor can also mediate endocytosis of IgM-coated bacteria and immune-complexes (41–43). IgM binding by PfEMP1 may therefore allow infected erythrocytes to interfere with these functions of Fcα/μR. We have recently shown that the binding of IgM to the Fcα/μR is dependent on the pentameric structure of IgM and that mAbs that inhibit binding to PfEMP1 also prevent binding of IgM to the Fcα/μR (46), suggesting that the binding site for the two ligands lie close to each other on IgM. Indeed, by using domain-swapped Abs, we demonstrated that binding involved both the Cμ3 and Cμ4 domains in the IgM polymer (and similar results were recently observed with FcμR (35)). However, the 80 kDa secretory component that shares homologous binding sites with Fcα/μR, does not block the binding of IgM to DBL4β, implying that IgM can accommodate the simultaneous binding of both ligands (16,46). That the interaction with Fcα/μR requires unique amino acid contacts in IgM is known, since domain swap Abs containing only Cμ4, while binding PfEMP1 do not bind Fcα/μR. Furthermore mouse IgM does not bind PfEMPI, yet is capable of binding both human Fcα/μR and FcμR, supporting our notion that unique contacts are involved in the binding of human IgM to either PfEMPI or the Fcα/μ–and Fcμ-receptors. Given the overlapping nature of the binding sites, it could be argued that this region is important for human IgM function, and that it is beneficial for the infected erythrocyte to manipulate the receptor's access to this region.

Interference with complement

The ability of IgM to agglutinate and neutralize pathogens so effectively is largely due to its polymeric structure that endows the molecule with increased avidity. This unique structure also makes IgM very effective at activating the classical complement cascade. A single molecule of IgM can trigger complement activation leading to the lysis of a single erythrocyte, an equivalent task requiring one thousand IgG molecules (37,47).

Binding of PfEMP1 at the interface of the IgM Cμ4 domain therefore offers an elegant explanation for our finding that C1q binding did not interfere with the DBL domain interaction (16), since the residues involved in C1q binding are found in the Cμ3 domain on the flat side of the IgM molecule (Fig. 4) (30). However, IgM binding by PfEMP1 may nonetheless interfere with complement activation by inducing conformational changes in the central core of IgM that may lock out the C1q binding sites on IgM. Alternatively, PfEMP1 binding may force IgM to be orientated with its flat side directly facing the red cell membrane, thereby making the C1q binding sites inaccessible to C1q.

If these hypotheses are correct C1q would rarely be found bound to infected erythrocytes. Although no published studies have reported the presence of C1q on infected erythrocytes, parasite-specific IgM and other complement factors including C3, C4 and C9 have been described on infected erythrocytes (48). However, these can all be deposited via the alternative or lectin pathways in a manner independent of either C1q or IgM. Although we know that DBL binding can occur to IgM opsonized onto haptenated erythrocytes, we still

do not know if PfEMP1 binding can occur after engagement of an infected erythrocyte by parasite-specific IgM, and what effect this might have on subsequent C1q docking. Human IgM mAbs against other knob-associated antigen(s) or non-IgM binding DBL domains would clearly be useful to address these questions.

Interference with the BCR

Infection with *P. falciparum* induces a hypergammaglobulinemia indicative of the presence of B cell mitogens (36). We have become intrigued by the possibility that these various DBL domains may interact directly with IgM^+ B cells through the B cell receptor (BCR), as Cμ4 specific mAbs that inhibit PfEMP1 binding to IgM have also been shown to augment S phase entry of human B cells via the IgM^+ BCR (16,49). IgM-mediated signaling is required for the development of a normal B-cell memory response (50). Our finding that malaria parasites, known to promote polyclonal B-cell activation, encode molecules specific for Cμ4 certainly suggests that binding to this region of the BCR might also be immunologically important and merits further investigation. Although most circulating infected erythrocytes will presumably be saturated in IgM, making them unable to simultaneously engage IgM^+ B cells through the same DBL domain, other regions of PfEMP1 may contact the IgM^+ BCR after the primary IgM contact. One candidate is the CIDR domain that is already known to interact directly with the B cell (51).

Evidence demonstrating the ability of the BCR to cluster could support the idea that PfEMP1 may bind to the Cμ4 of the BCR and to soluble pentameric IgM via similar mechanisms. It is well known that BCR mutants missing the Cμ4 domain are unable to inhibit signaling upon Ag binding, and that Cμ4 domains expressed alone on the B-cell surface cluster spontaneously to activate the cell (49,52,53). Therefore the DBL domain may cross-link two BCR monomers (as in the model with pentameric IgM (Fig. 2 and Fig. 3), or binding may induce conformational changes in the BCR revealing an interface that promotes BCR clustering with subsequent uncontrolled activation. Coincidently, the BCR governs the sub-cellular location of Toll-like receptor 9 (TLR9), a ligand for *Plasmodium* DNA that leads to hyper responses to DNA-containing antigens (53,54). Whether the FcμR, potentially as a co-receptor at the surface of the B-cell plays a role in BCR function and memory induction, and the potential role for interference by malaria parasites through IgM binding now needs to be investigated, perhaps in FcμR deficient mouse models of malaria.

Interference with endothelial receptors

Sequestration of infected erythrocytes in the microvasculature and the placenta is a cardinal feature of *P. falciparum* infection, and it is thought to benefit the parasite by allowing infected erythrocytes to avoid being removed by the spleen (27). It is possible that IgM bound to infected erythrocytes could cross-bridge to host IgM-binding receptors on endothelial or placental cells to promote sequestration of parasitized cells. It is currently unknown if any of the IgM receptors discussed above are expressed on human endothelial cells in the microvasculature or on placental syncytiotrophoblast cells. One intriguing recent finding is that CD300LG (also known as CLM9, TREM4 or NEPMUCIN) shares 35% identity with the Fcα/μR, and is expressed on capillary endothelium particularly in placental tissues (55). Although CD300LG is a functional receptor for L-selectin mediated lymphocyte rolling (56), it has also recently been shown to bind IgM (55). Future experiments should investigate the expression patterns of IgM binding proteins in the human microvasculature and placenta, and address the possibility that IgM (be it natural or parasite-specific) bound to infected erythrocytes could cross-bridge to promote sequestration.

Interference with neutralizing and adhesion blocking antibodies

Rosetting and placental parasite isolates may also bind non-specific IgM to allow masking of critical PfEMP1 domains from the destructive action of specific Abs e.g. IgG. This 'umbrella' hypothesis is supported by the observation that the domain adjacent to the IgM binding domain in *var2csa* appears to be the only domain inducing highly adhesion blocking antibodies in humans. The hypothesis is readily testable using domain specific and parasite adhesion blocking anti-*var2csa* IgG (57).

Conclusions

The ability of diverse *P. falciparum* isolates to bind a conserved region of the IgM Fc suggests that this function is essential to parasite survival. A molecular understanding of how malaria parasites bind the Fc of both natural and specific IgM may therefore lead to the development of novel inhibitors of this interaction. For example, small molecule inhibitors may prevent docking of PfEMP1 to either IgM or the BCR and prevent hypergammaglobulinemia, or in the case of immunity, lead to the development of greater memory responses when co-injected with vaccines. Understanding how infected erythrocytes manipulate IgM may also lead to the development of improved adjuvants based on the remarkable properties of this fascinating molecule (12,14). DBL-based reagents, like those already developed for bacterial protein A and G, may be used for the specific detection and purification of human IgM. As such they promise to become valuable tools for structural and functional studies of IgM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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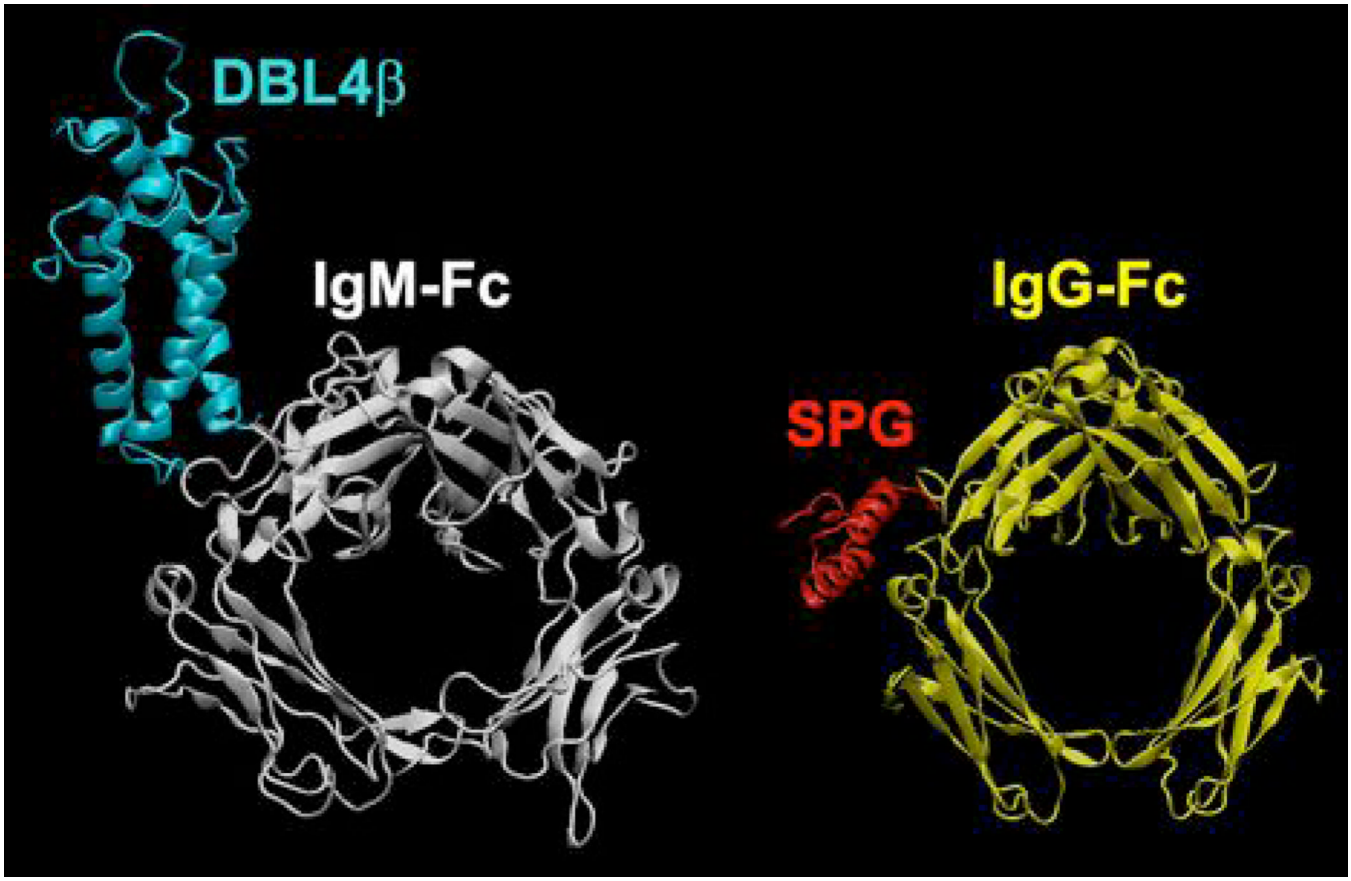

Fig 1. Contact areas of Fc with pathogen Fc-binding proteins

Ribbon diagrams of the IgM-Fc in complex with the DBL4β domain of the TM284var1 variant of PfEMP1 (left) and the IgG-Fc in complex with domain C2 of Staphylococcal Protein G (right). The structure of the IgM-DBLβ complex is described in Fig. 1. Both proteins bind at analogous positions in the inter-domain region of the immunoglobulin Fc.

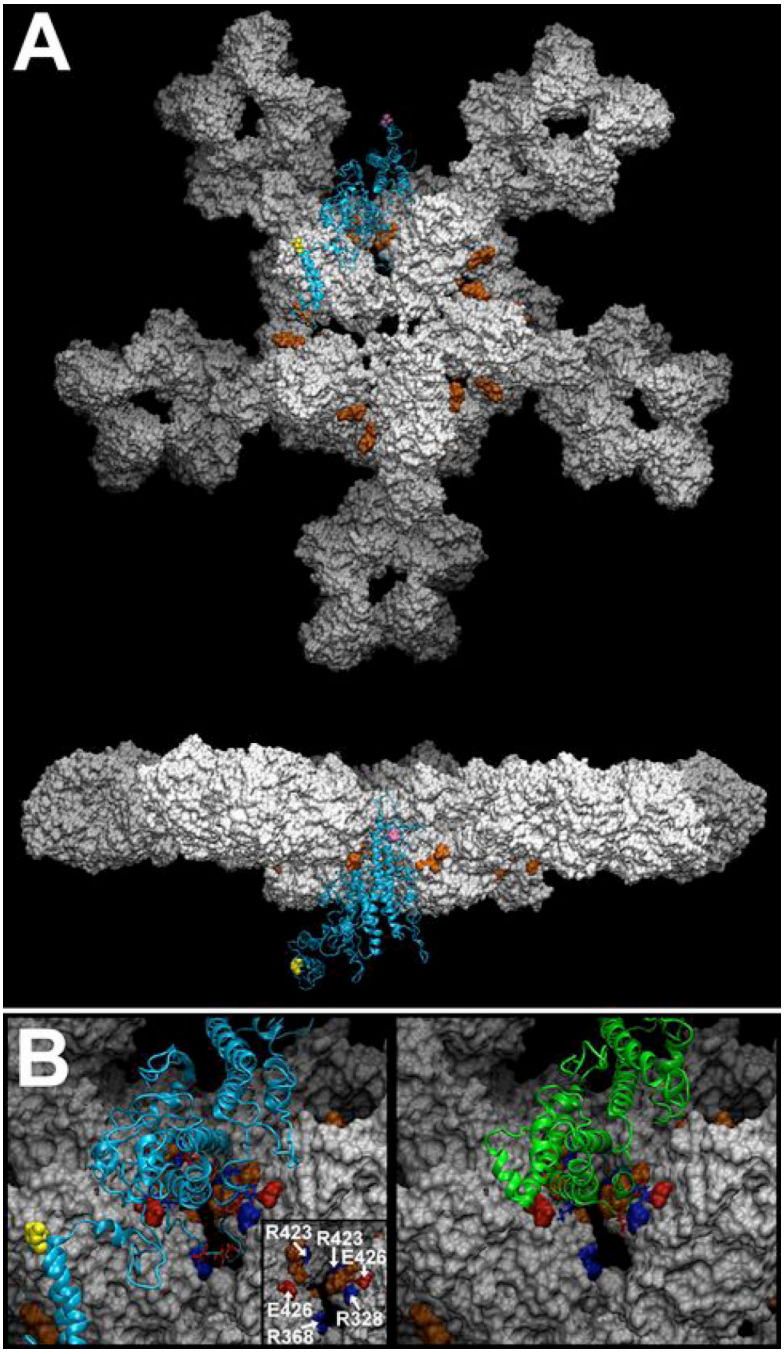

Fig. 2. Homology-based model of the lgM-DBL4β complex

(A) The mushroom-shaped structure of IgM (white) is based on the known bent structure of IgE and direct cryo-AFM images (described in ref 30), and was left unaltered in the modelling with DBL4β. The novel homology model of TM284var1 DBL4β (blue) shown here is based on the known structures of members of the DBL family (31,32,60,62–64). The DBL4β domain was manually docked onto the IgM structure, taking heed of constraints described in the text. The top and middle panels show views of the lgM-DBL4β complex looking down onto the “protruding”-side of the IgM molecule and from the side, respectively. The residues in IgM identified in mutational analyses to be critical for binding to TMR284var1 DBL4β (Pro^{394}-Pro^{397} and Pro^{444}-Pro^{447} of the Cμ4 domain) are shown in

orange (16). (B) Regions within sub-domain 2 of TM284 DBL4β (blue) and FCR3 DBL6ε (green) domains that directly interact with IgM (white). A pocket of charged residues found at the monomer-monomer interface of IgM (boxed inset) is directly adjacent to oppositely charged residues found in the DBL domain loops, suggesting a mechanism of association between members of the DBL protein family and human IgM molecules. Notice that the modeled TM284 DBL4β domain in this region has a much longer loop than in the FCR3 DBL6ε domain, yet this loop is found to be easily accommodated within the DBL-IgM interface, and with this interaction, furthers the apparent charge complementarity at this DBL-IgM interface.

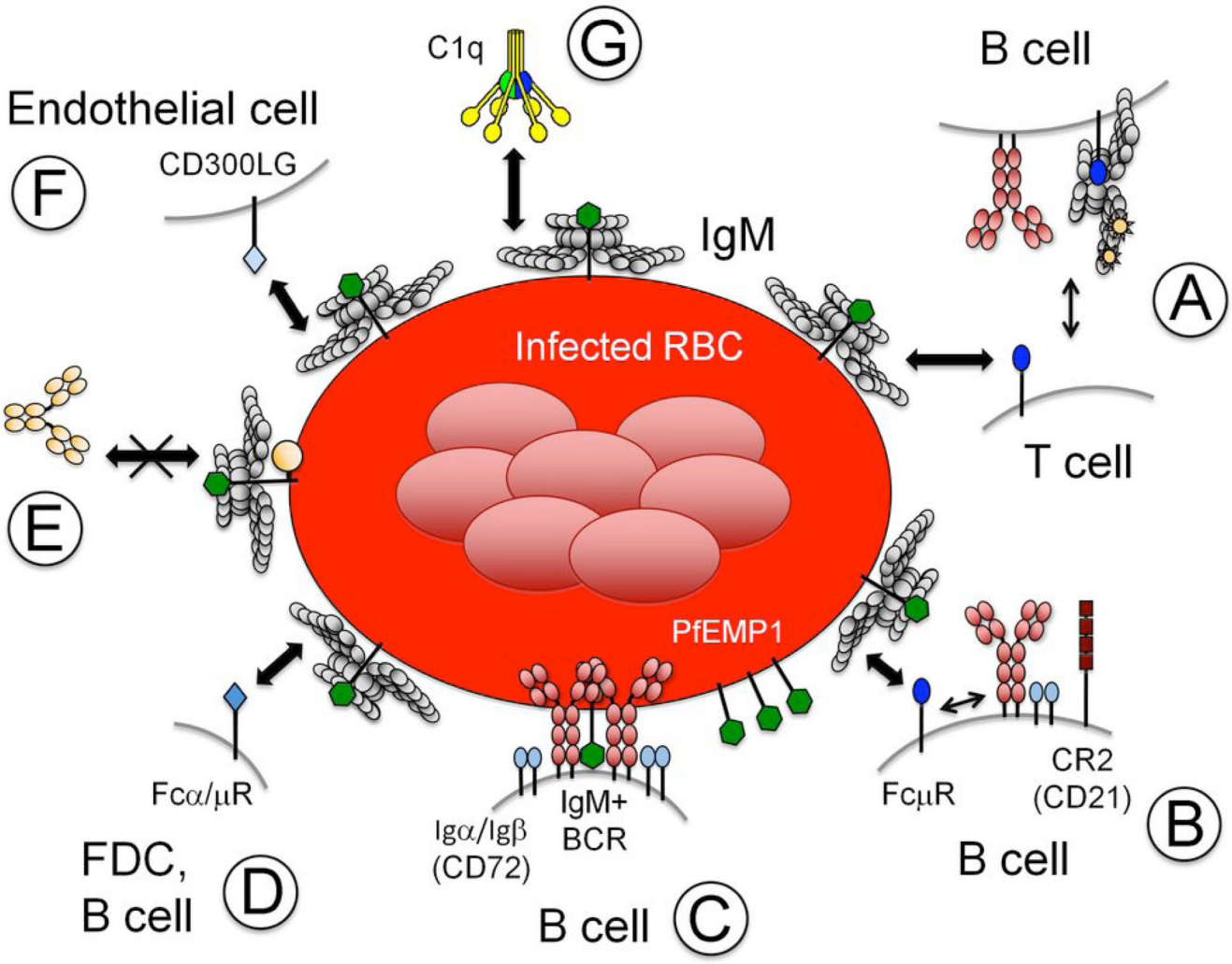

Fig. 3. Possible functional consequences of the PfEMP1-IgM interaction

Shown are several potential IgM-effector associations that could be altered as a result of the known DBL-IgM interaction. As described more fully in the text, these include those with the recently identified FcμR protein found both in T cells, where it could play a role in mediating immunological synapse formation (A), and in B cells, where it is possibly involved in activating these cells through interactions with BCR (and CR1/2 (B)); the Fcα/μR protein predominantly localized to FDCs (D); the endothelial receptor, CD300LG (F); and the potent activator of the classical complement pathway, C1q (G). Also depicted in the figure are the possibilities for direct interactions between PfEMP1 proteins and BCR complexes in B cells, which could promote activation of these complexes through clustering (C), and a blocking of potential antigens on infected RBCs as a result of a "shielding" mechanism of IgM bound to PfEMP1 (E).

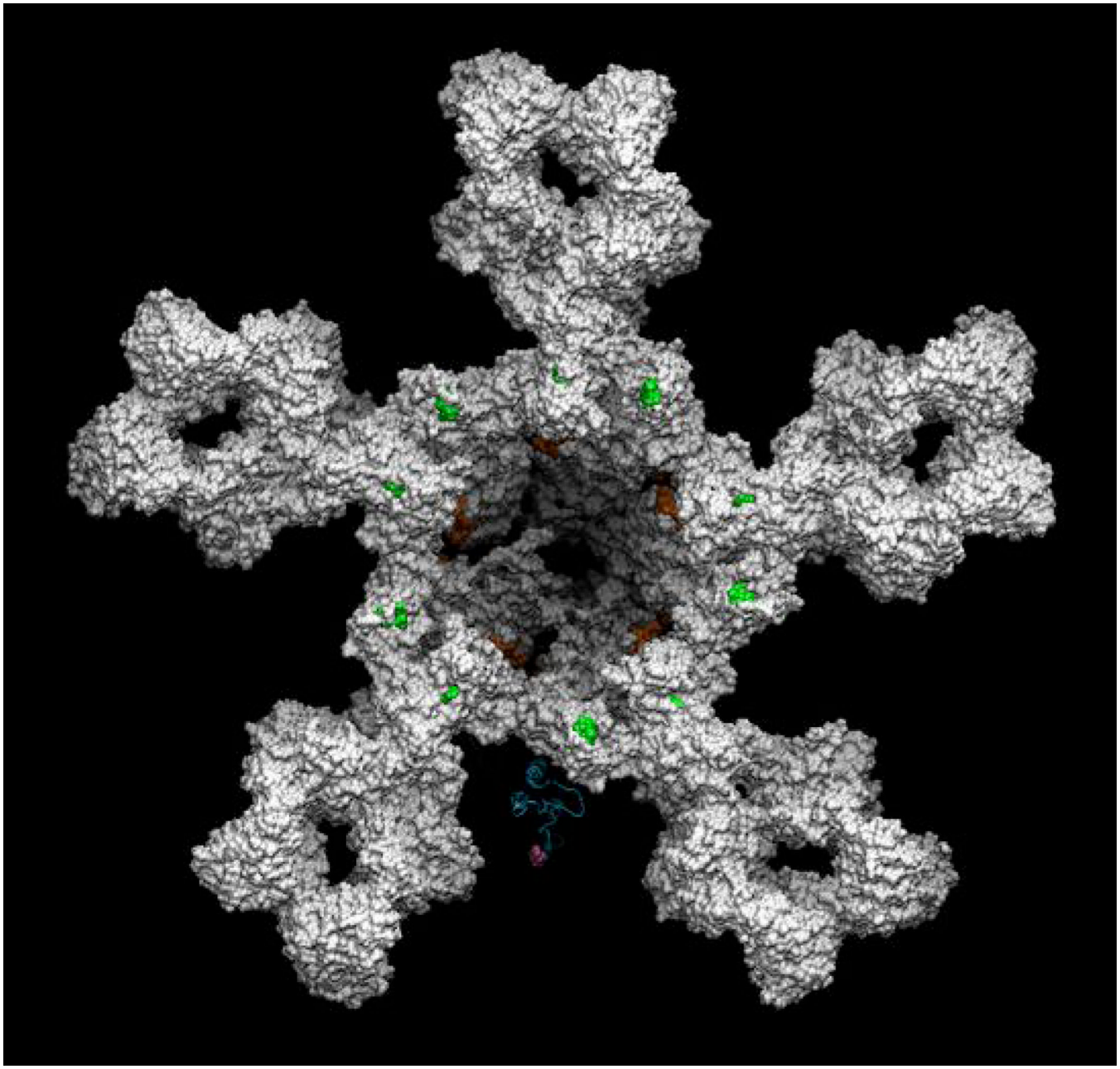

Fig. 4. C1q-binding sites in the lgM-DBL4β complex

The image shows the lgM-DBL4β model looking down onto the “flat”-side of the IgM molecule, where the putative C1q binding sites on IgM (green) are located (30). It is clear that C1q would not be expected to sterically interfere with DBL4β binding to IgM, consistent with experimental observations (16).

Table 1

Known IgM binding DBL domains from*P. falciparum*

PfEMP1 variant	Domain	Reference
FCR3S1.2*var*1	CIDR	58
TM284S2*var*1	DBL2β	59
FCR3*var*1 *csa*	DBL7ε	60
FCR3*var*2*csa*	DBL6ε	60
3D7*var*2*csa*	DBL2-X, DBL5ε, DBL6ε	61
TM284*var*1	DBL4β	16

Table 2

Amino acid residues in DBL domains predicted from molecular modelling to interact with IgM

TM284*var1* DBL4β	**FCR3 *var2csa* DBL6ε**	***3D7var2 csa* DBL5ε**	**FCR3*var1csa* DBL7ε**
Lys302	Arg2494	None	None
Glu299	Glu2491	Asp2149	Glu3082
Arg284	Arg2481	Lys2141	Lys3072
Glu183	None	Glu2079	Glu3005
Glu185	None	Asp2080	Glu3007